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13. ABSTRACT (Maximum 200 Words)
The purposes of this study are to provide quantitative estimates of 1) the effective amount of vitamin D produced in the skin as a function of skin pigmentation; and 2) the rate of utilization of vitamin D as a function of ethnicity. The outcome will be estimates of the amount of vitamin D that must be given orally to military personnel of different races and in different assigned locations so as to ensure and maintain normal vitamin D status. In the first 15 months' work (the period covered by this report), we have accumulated about 50% of the targeted measurements for both objectives, in a racially diverse sample. No quantitive results will be available until all the measurements have been made and analyzed as a unit

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INTRODUCTION

The purpose of this project is to develop quantitative estimates of 1) the amount of vitamin D produced by skin exposure to sunlight, and 2) the amount of oral vitamin D that must be given to supplement solar inputs so as to achieve desired vitamin D levels in military personnel of differing races and skin pigmentation.

This is the second annual report with respect to the above-referenced award. Although the award was made as of 1 October 2001, authorization to proceed was not received from USAMRMC until 15 July 2002. Hence this report, although technically covering the first two years of the award, describes work performed only from 15 July 2002 until submission of this report, i.e., a period of roughly only 15 months.

BODY OF REPORT

Logistics. As reported last year, upon authorization to proceed, we began immediately to finalize the procedure manual and to recruit a project manager, one preferably of minority background with good community contacts. We ultimately selected Lisa Auberry-Adams and began immediately the process of having her complete the University IRB training program required of all personnel involved in human subjects' research. At the same time, the principal investigator and Osteoporosis Research Center staff began the process of recruitment of subjects for Experiment 2 (which measures subjects at the end of a summer of outdoor sun exposure and then again five months later after a winter of no significant sun exposure). We just barely had time, in the few weeks available following authorization, to get this component launched. We also acquired an electronic skin color reflectance meter [SmartProbe 400, Innovative Measurement Solutions, Inc., Milford CT] to provide an objective, reproducible measure of the three principal contributors to skin tone and to the change therein induced by sun exposure. This instrument had to be calibrated, its reproducibility determined, and standard operating procedures for its use developed, so that it could be deployed in the subjects then enrolled in Experiment 2.

Work Performed: Experiment 2 – First Phase. The purpose of Experiment 2 is to quantify the serum 25(OH)D response (and its physiological correlates) to summer sun exposure in persons with a wide range of skin pigmentation. As of 30 September 2002 we had enrolled 38 individuals and had obtained the first (i.e., late summer) measurements as specified for Experiment 2. Second visits were scheduled for February 2003. This number (38) was just shy of our target of 40 participants and the shortfall is due to the shortage of time between authorization and the closing of the window of opportunity for enrolling summer workers. We planned to make up the difference in the current year's work plan. Thirty-four of those 38 individuals returned for the February (late winter) visit. The ratio and sex breakdown of the group completing the first phase of Experiment 2 is as follows:

	Non-Hispanic Caucasian	Other	African-American	Totals
Male	9	0	11	20
Female	6	1	7	14
Totals	15	1	18	34

For each of these subjects we have obtained the suite of specimens/measurements specified in the approved protocol, i.e., history of sun exposure by duration and clothing type; skin pigmentation by reflectance meter measurement; calcium absorption efficiency; measurements of the full set of hormones regulating the calcium economy [i.e., PTH, $1,25(OH)_2D_3$, 25(OH)D], as well as blood vitamin D levels themselves, urine calcium excretion, and bone densitometry.

Work Performed: Experiment 2 – Second Phase. As of 30 September 2003, we had enrolled an additional 28 individuals and had obtained the initial set of measurements (i.e., the late summer set). This is 12 short of the targeted figure of 40. (See below for our plan to catch up.) The reason for the shortfall was that the project manager employed a year earlier left us on 15 August 2003, precisely at the time when recruitment for this project component was to have shifted into high gear. It has taken us until October 20 to find and train a suitable minority professional to replace her. This person, Tamicka Bradley, B.S.N., is now aboard but too late to have helped in recruitment for the summer sun study (Experiment 2). Only by a joint effort of several Osteoporosis Research Center project staff have we been able to recruit the 28 new individuals for this phase of Experiment 2.

The sex and ethnic breakdown of the total now enrolled in, and potentially completing, Experiment 2 is as follows:

	Non-Hispanic Caucasian	Other	African-American	Totals
Male	18	2	15	35
Female	13	4	10	27
Totals	31	6	25	62

Work Performed: Experiment 1 – First Phase. The purpose of Experiment 1 is to quantify the ethnic differences (if any) in rate of metabolism of known inputs of vitamin D_3. It is designed to be executed over the winter months when solar vitamin D input is minimal and total input can be controlled by the investigators through daily oral dosing of controlled quantities of vitamin D_3. Our plan was to split the project into two phases, studying doses of zero and 1000 IU/d during Phase 1 (performed this past year) and doses of 5,000 and 10,000 IU/d during Phase 2 (beginning at the time of preparation of this report).

During Phase 1, completed last Spring, we entered 46 subjects (target 40), 17 African-Americans, 5 Hispanics, and 14 non-Hispanic whites. Twenty-three were randomized to a zero vitamin D dose, and 23 to 1000 IU/d (target: 20 for each dose). Blood samples were drawn at intervals of approximately four weeks, starting with a zero-time sample. Sera were analyzed for vitamin D, 25(OH)D, and PTH. The fully quantitative data relating dose to response will be derivable only after work on all four dosage groups has been completed. However, we can report that the zero-dosage group had a fall in serum 25(OH)D from an average of 43.7 to 38.7 nmol/L. Both values are well below desirable levels (~80 nmol/L) and the extent of the drop over winter is about what would be expected.

The group receiving 1000 IU/d, by contrast, had a rise in serum 25(OH)D, from an average of 42.6 to 90.0 nmol/L. This is a larger rise than we had previously observed in an exclusively white group of subjects at this dose, but the starting value was lower in this study, and these results may be more applicable to a predominantly black and Hispanic population. The finding of this difference constitutes validation of the need to study African-Americans in addition to Caucasians. Definitive conclusions in this regard must wait until we have data for all four dosage groups and a sufficiently large sample to permit separate analysis by race.

Work Plan for the Forthcoming Year. We have already begun the recruitment for Experiment 1, Phase 2 (the winter vitamin D dosing component). We anticipate reaching our target enrollment by mid November and will have preliminary results at the time of next year's annual report. We will also be bringing back the second batch of subjects enrolled in Experiment 2, Phase 2,. (The summer sun exposure component) for their end of winter visit. We are completing analyses of the specimens obtained from them at their end-of-summer visit, but have no results to report at this time. Also, in order to compensate for the shortfall in enrollment in Experiment 2, we will deploy a revised strategy – recruiting likely summer workers at the end of February for their end-of-winter measurement, and then will plan to bring them back for their end-of-summer visit six months later. That will save six months' time, but is likely to have a lower yield of completers because outdoor summer work is not always a certainty for workers at the end of winter. We will plan to over-recruit so as to compensate for that eventuality.

KEY RESEARCH FINDINGS

Chemical analyses of the various hormone levels have been performed on the subjects of Phase 1 during the –02 year of the award. A portion of these results were presented in poster form at the meeting of the American Society for Bone and Mineral Research in Minneapolis, September 20, 2003. Copies of the poster and the associated Abstract are attached as Appendix I. Full analysis and publication must wait completion of all the subjects in this Experiment (n = 80) in subsequent years of the project.

REPORTABLE OUTCOMES

As noted in the foregoing, the reportable outcomes from this study will consist of 1) best quantitative estimates of skin production of vitamin D as a function of skin pigmentation and extent of skin exposure; and 2) best quantitative estimates of rate of utilization of vitamin D_3 as a function of race/ethnicity. Taken together, both will yield estimates of the quantity of vitamin D that must be given to military personnel to ensure maintenance of desired vitamin D status. Since much of the work is still to be done, those quantitative estimates have not yet been derived, and hence there are as yet no reportable outcomes relating to the primary objectives of the project. However, secondary findings are available and doubtless further such will develop as we accumulate more measurements. An example of such secondary data can be found in the Abstract and Poster enclosed as Appendix I.

CONCLUSIONS

None to date (see above).

REFERENCES

1. Barger-Lux MJ, Auberry-Adams L, Lappe JM, Recker RR, Heaney RP. Towards quantifying the relationship of constitutive skin color to daily skin dose of vitamin D_3 in healthy adults with ample summer sun exposure. *J Bone Miner Res* 18 (Suppl 2):S180, 2003.

APPENDIX I

Towards quantifying the relationship of constitutive skin color to daily skin dose of vitamin D_3 in healthy adults with ample summer sun exposure. M.J. Barger-Lux, L. Auberry-Adams*, J. M. Lappe, R.R. Recker. and R.P. Heaney, Creighton University, Omaha, NE

We report here preliminary results of work to quantify the relationship of inherent skin pigmentation and summer increment of vitamin D_3 among healthy adults with ample summer sun exposure and limited non-solar sources of vitamin D. The 38 subjects (aged 20 to 45 yr) classified themselves by race as black (n=20), white (n=15), or other (n=3). Data were gathered in late summer (Aug. 29 to Sept. 21). We estimated extent (% body surface area) and duration (hr/wk) of summer sun exposure by interview. We also determined BMI; fasting serum 25(OH)D, vitamin D_3, $1,25(OH)_2D$, PTH, and Ca; fasting urine Ca-to-creatinine ratio; and Ca absorption fraction.

We used a portable colorimeter (SmartProbe, IMS Inc., Milford, CT) that utilizes the CIE L*a*b* color system to measure constitutive skin color of the upper inner arm. There was a strongly positive curvilinear relationship (R^2=0.5234) between the "L" readings (a continuous darker-to-lighter scale) and 25(OH)D. The lowest and highest "L" tertiles (i.e., darkest and lightest subgroups) differed significantly in 25(OH)D (47.6 ± 12.5 vs 91.9 ± 17.3 nmol/L); vitamin D_3 (0.41 ± 0.31 vs 2.87 ± 2.39 ng/mL; fasting urine Ca-to-creatinine ratio (0.033 ± 0.026 vs. 0.121 ± 0.096 g/g); and sun-exposed body surface area (28.1 ± 8.1 vs 40.1 ± 12.6%); They did not differ in $1,25(OH)_2D$, PTH, serum Ca, or Ca absorption fraction.

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Toward Quantifying the Relationship of Constitutive Skin Color to Daily Skin Dose of Vitamin D_3 in Healthy Adults with Ample Summer Sun Exposure

Creighton UNIVERSITY Medical Center

M. Janet Barger-Lux, Lisa Auberry-Adams,* Joan M. Lappe, Robert R. Recker, and Robert P. Heaney, Creighton University, Omaha, Nebraska, USA

INTRODUCTION

We report here preliminary results of work to quantify the relationship of constitutive (inherent) melanin skin pigmentation and summer increment of 25-hydroxyvitamin D (1). This study extends earlier work (2,3) to subjects with a wide range of skin tones.

SUBJECTS and METHODS

Subjects

Subjects were ostensibly healthy adults living in our city at 41.2° north latitude. They had ample summer sun exposure and limited non-solar sources of vitamin D. The 34 subjects who completed the study, including self-classifications by race, were as follows:

	Black	White	Other	Totals
Women	7	6	0	13
Men	11	9	1	21
Totals	18	15	1	34

Protocol

VISIT 1: Aug. 29-Sept. 21, after summer sun exposure
VISIT 2: Feb.1-Mar. 15, after winter sun deprivation

- Height, weight, melanin skin pigmentation, 25(OH)D, $1,25(OH)_2D$, vitamin D_3, PTH, fasting serum Ca, fasting urine Ca:creatinine, and Ca absorption fraction
- Duration (hr/wk) and extent (sun-exposed body surface area) of summer sun exposure; calculated Sun Index (Visit 1 only)
- Body composition (Visit 1 only)

Methods

SUN-EXPOSED BODY SURFACE AREA. We adapted the "rule of nines" to estimate the fraction of BSA exposed by each subject's usual outdoor attire (2,4).

SUN INDEX. We computed this variable to combine duration (hr/wk) and extent of summer sun exposure:

Sun Index = usual sun exposure x sun-exposed BSA

25-HYDROXYVITAMIN D. Competitive binding assay, ^{125}I-labeled ligand (Nichols Institute Diagnostics, San Clemente, CA.) Each subject's late summer less late winter value was used to estimate summer increment.

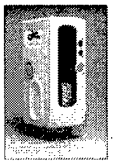
OTHER TESTS. Methods as described earlier (2).

MELANIN PIGMENTATION. We measured skin tone by use of a portable tristimulus colorimeter, the IMS Smart-Probe-400 (IMS Inc., Milford, CT; see Fig. 1). The device uses a standard color system in which the "L" or "lightness" axis (black-white) is a measure of melanin skin pigmentation (5). For each subject, we determined average "L" readings at an unexposed site (upper inner arms at Visit 2), as a measure of constitutive (inherent, untanned) melanin pigmentation.

Figure 1.
IMS SmartProbe-400 and Human Skin Tone Chart

We used this portable handheld colorimeter to measure skin tones. The panel of standards spans the colorspace of interest.

Photos are courtesy of IMS Inc., Milford, CT.



Human Skin Tone Chart
Mean "L" Readings
(black-white color axis)

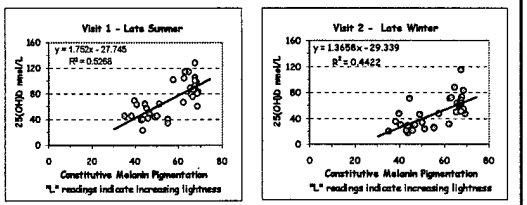
Std 1 67.0	Std 2 61.1	Std 3 56.5
Std 4 54.5	Std 5 48.7	Std 6 44.4
Std 7 37.8	Std 8 36.8	Std 9 34.3

RESULTS

Table 1. Subjects at Visit 1. Late-summer data as median and inter-quartile range, n = 34.

Age (yr)	35.8	(32.9 - 40.9)	Serum Ca (mg/dL)	9.4	(9.2 - 9.8)
BMI (kg/m2)	28.7	(25.6 - 31.8)	Urine Ca:creat (g/g)	0.064	(0.033 - 0.092)
Body comp (fat/total)	0.255	(0.188 - 0.322)	Ca absorption fraction	0.308	(0.261 -0.372)
25(OH)D (nmol/L)	67.3	(45.4 - 91.4)	Summer sun exposure		
Vitamin D_3 ng/mL	0.3	0.3 - 3.1	Duration (hr/wk)	27.5	(17.5 - 40.0)
PTH pg/mL	33.9	24.3 - 50.1	Sun-exposed BSA	0.37	(0.25 - 0.45)
$1,25(OH)_2D$ pg/mL	32.9	30.6 - 39.0	Sun Index	9.5	(6.3 - 13.9)

Figure 2. Constitutive Melanin Pigmentation and 25(OH)D by Season



RESULTS - continued

Table 2. Tests of Difference by Constitutive Melanin Pigmentation.
Mann-Whitney tests of difference between lowest and highest "L" tertile

	Darkest Tertile "L" readings <48.6 median	Darkest Tertile "L" readings <48.6 inter-qtl range	Lightest Tertile "L" readings >66.1 median	Lightest Tertile "L" readings >66.1 inter-qtl range	P value
Sun-exposed BSA	0.29	0.25 - 0.41	0.45	0.26 - 0.45	<0.1
At Visit 1					
Age	39.4	34.8 - 43.7	33	28.6 - 36.2	<0.02
25(OH)D nmol/L	45.6	39.7 - 62.5	87.5	80.6 - 99.4	<0.001
Vitamin D_3 ng/mL	0.3	0.2-3 - 0.3	3.1	0.7 - 5.2	<0.005
Urine Ca:creatinine (g/g)	0.032	0.013 - 0.058	0.079	0.072 - 0.235	<0.001
At Visit 2					
25(OH)D nmol/L	28.3	20.4 - 33.9	60.4	51.3 - 72.5	<0.001

Tests of other variables failed to disclose corresponding differences.
Analyses of Vitamin D_3 at Visit 2 were unavailable.

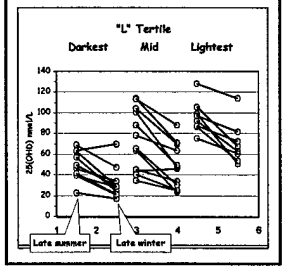
Table 3. Vitamin D Status by Season and Constitutive Melanin Pigmentation

	"L" tertiles Darkest	Mid	Lightest
Late Summer 25(OH)D			
<37.5 nmol/L	9%	8%	0%
37.5 - 79.9 nmol/L	91%	50%	18%
80+ nmol/L	0%	42%	82%
Totals	100%	100%	100%
Late Winter 25(OH)D			
<37.5 nmol/L	82%	42%	0%
37.5 - 79.9 nmol/L	18%	50%	82%
80+ nmol/L	0%	8%	18%
Totals	100%	100%	100%

"L" readings indicate increasing lightness

Osteoporosis Research Center

Figure 3. Seasonal Changes in 25(OH)D by Constitutive Melanin Pigmentation



COMMENT

1. In darker-skinned persons, ample summer sun exposure at a temperate latitude fails to
   - produce robust late-summer 25(OH)D levels
   - protect against low late-winter 25(OH)D levels
2. This topic is of interest because:
   - vitamin D probably helps to prevent several serious health problems (e.g., 6) in addition to osteoporosis
   - recommendations for vitamin D supplementation should probably vary by locale, opportunity for sun exposure, and skin color
3. Measurement of skin color using a standard colorimetric system is useful for studying differences in vitamin D response to sun exposure.

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